11. See, for example, B. R. Clarridge, L. L. Sheeky, T. S. Hauser, in *Sociological Methodology, 1978*, K. F. Schuessler, Ed. (Jossey-Bass, San Francisco, 1977), pp. 185-203.
12. L. M. Terman, Ed., *Genetic Studies of Genius* (Stanford Univ. Press, Stanford, Calif., 1925-1959), vols. 1 to 4.
13. U.S. Department of Labor, *Manpower Research Monograph No. 30* (Government Printing Office, Washington, D.C., 1974).
14. R. W. Fogel and S. L. Engerman, *Time on the Cross* (Little, Brown, Boston, 1974); H. G. Gutman, *Slavery and the Numbers Game* (Univ. of Illinois Press, Urbana, 1975).
15. E. O. Wilson, *Sociobiology* (Harvard Univ. Press, Cambridge, Mass., 1975).
16. J. Hirshleifer, *J. Law Econ.* **20**, 1 (1977).
17. K. J. Arrow and F. H. Hahn, *General Competitive Analysis* (Holden-Day, San Francisco, 1971).
18. H. A. Simon, *Am. Econ. Rev.* **69**, 493 (1979).
19. M. Friedman, *Essays in Positive Economics* (Univ. of Chicago Press, Chicago, 1953), pp. 16-30.
20. To a very close approximation the logarithms of the sizes of business firms vary linearly with the logarithms of their rank in the industry or economy—a regularity usually known in economics as the Pareto Law, and observed also in a wide variety of other social phenomena (for example, city size distribution). See Y. Ijiri and H. A. Simon, *Skew Distribution and the Sizes of Business Firms* (North-Holland, Amsterdam, 1977).
21. These phenomena provide an excellent example of the importance of having hard data against which to check widely held popular beliefs. Contrary to popular belief, the data show that large companies do not grow, on average, more rapidly than small companies. A. Singh and G. Whittington, *Growth, Profitability and Valuation* (Cambridge Univ. Press, Cambridge, 1968); J. Steindl, *Random Processes and the Growth of Firms* (Hafner, New York, 1965).
22. R. R. Nelson and S. G. Winter, *Bell J. Econ.* **9**, 524 (1978).
23. For a wide-ranging discussion of the issues of this section see *Behav. Sci.* **24**, whole No. 1 (1979); also see Wilson (*15*).
24. The definitive treatment is L. J. Savage, *The Foundations of Statistics* (Wiley, New York, 1954).
25. D. Kahneman and A. Tversky, *Psychol. Rev.* **80**, 237 (1973).
26. G. E. Box, *Bayesian Inference in Statistical Analysis* (Addison-Wesley, Reading, Mass., 1973).
27. H. Raiffa, *Decision Analysis* (Addison-Wesley, Reading, Mass., 1968).
28. J. J. Moder and S. E. Elmaghraby, Eds., *Handbook of Operations Research* (Van Nostrand-Reinhold, New York, 1978), vols. 1 and 2; H. M. Wagner, *Principles of Operations Research* (Prentice-Hall, Englewood Cliffs, N.J., ed. 2, (1975); see also H. J. Miser, *Science* **209**, 139 (1980).
29. J. von Neumann and O. Morgenstern, *The Theory of Games and Economic Behavior* (Princeton Univ. Press, Princeton, N.J., 1944).
30. A. Rapoport and A. M. Chammak, *Prisoner's Dilemma* (Univ. of Michigan Press, Ann Arbor, 1965).
31. R. Radner, personal communication.
32. An excellent example of recent research in this domain is J. C. Harsanyi, *Rational Behavior and Bargaining Equilibrium in Games and Social Situations* (Cambridge Univ. Press, Cambridge, 1977).
33. K. J. Arrow, *Social Choice and Individual Values* (Wiley, New York, 1951).
34. The first volume of *Cognitive Science* was published in 1977. Papers of the first National Conference on Cognitive Science will be published in the 1980 volume.
35. J. R. Anderson, *Language, Memory and Thought* (Erlbaum, Hillsdale, N.J., 1976); H. A. Simon, *Models of Thought* (Yale Univ. Press, New Haven, Conn., 1979).

Frontiers of the Biological Sciences

Bernard D. Davis

Summary. The history of the molecular revolution in biology is described, emphasizing its dependence on the emergence of bacterial genetics, the fusion of genetics and biochemistry, and the development of greatly improved techniques for studying macromolecules. Central concepts have included molecular information transfer, both by nucleic acids and by allosteric proteins; the spontaneous conversion of one-dimensional information into three-dimensional structures; and the extraordinary unity in the molecular mechanisms underlying the rich diversity of biology. The merging of molecular and morphological studies, to yield the very broad field of cell biology, is described more briefly, as are also some present frontiers in several areas of biology that present challenges at other levels of organization.

Those of us who were entering biology in the 1930's were very much encouraged by the essays of J. D. Bernal and J. B. S. Haldane, who predicted that the age of biology would soon emerge. Equally confidently, these authors predicted a similar success of scientific planning in solving the problems of economic and political organization. Little could the students of my generation foresee that biology would mature so rapidly, while the predicted social utopia would become more distant than ever.

The author is Adele Lehman Professor of Bacterial Physiology, Harvard Medical School, Boston, Massachusetts 02115. Please do not request reprints.

Having had such a dismal experience with the crystal ball, I shall concentrate in this article not on predicting the future but on characterizing the recent revolution in biology. In its vanguard was a group of bold investigators, in whose hands the formal entities and processes of genetics became concrete substances and reactions. The resulting field, generally called molecular biology, has focused on the detailed analysis of structures and interactions in a range of dimensions that had long been terra incognita, lying between the small molecules of the biochemist and the visible structures of the morphologist. These advances depended heavily on the development of techniques that greatly simplified and refined the analysis of macromolecules, and on the development of an industry to supply the required instruments and materials.

One consequence has been a heightened sense of the unity of biology, in terms of the universal features developed early in evolution and also in terms of the continuity between molecular and morphological structure. An even more profound consequence has been a deep understanding of the mechanisms that give living systems their unique capacity to function and to grow, by accumulating negative entropy at the expense of increased entropy in the surrounding environment.

Although physicists had expected to find novel physical laws underlying this property, only the same old physical forces were encountered—but they were found to be organized in novel ways that yield molecular information storage and a flow of information. Thus DNA stores a program, like a computer, on a one-dimensional tape; and cells use this information to specify the structures of their working machinery. Moreover, this machinery includes regulatory proteins, whose flow of information about the state of the cell and its surroundings directs the flow of material and energy. The foundation of these insights was already implicit in the early term organism, with its emphasis on organization. The idea of biological information also parallels modern advances in electronics and information theory, but the interactions have not been substantial.

With these developments the aim of

0036-8075/80/0704-0078$02.00/0

biochemical studies was no longer restricted to analysis of chemical mechanisms. One could also unequivocally define the biological function of an enzyme in the life of a cell, both by its regulatory responses and by the consequences of its alteration by mutation. Accordingly, teleonomy—the assumption that any normal structure or function has a "purpose" selected by evolution—became respectable in biochemistry, as it had long been in physiology and in evolutionary biology.

Apart from molecular biology, I shall discuss more briefly some highlights of several other fields, at higher levels of organization, whose frontiers are growing vigorously and present major challenges; these include cell biology, developmental biology, neurobiology, and evolutionary biology. Except for the discussion on the nervous system I shall have to omit altogether many lively areas of research on the specialized structures and functions of different organisms.

Microbial Genetics and the Origins of Molecular Genetics

A direct relation between genes and enzymes first attracted wide attention in 1940, when Beadle and Tatum showed that alterations in various genes in the mold *Neurospora crassa* resulted in loss of a corresponding biosynthetic enzyme. These mutants (and similar ones in bacteria) had the further useful property of accumulating the precursor of the blocked reaction, an intermediate normally present only in undetectable traces. With this approach, supplementing more conventional biochemical tools [enzyme fractionation, the incorporation of radioactively labeled substrates, and energy transfer from exergonic to endergonic reactions by adenosine triphosphate (ATP)], the territory of intermediary metabolism could be mapped. The results provided a background that was necessary for the later recognition of the intricate network of regulatory interactions in a cell.

But despite this fusion of genetics and biochemistry, the gene was still only a formal unit. Avery, MacLeod, and McCarty broke this barrier in 1944 by showing that the DNA from a virulent strain of the pneumococcus could *transform* an avirulent strain, that is, could endow it with the heritable ability to make a type-specific polysaccharide required for virulence. This discovery initiated the study of molecular genetics, but its profound significance was only gradually recognized, and it was not rewarded with a Nobel Prize (although Avery lived for 11 more years). One reason for the delayed impact was the absence of any known connection between bacteria and genetics at that time; these tiny cells were not believed to have a chromosome or to be capable of exchanging genes. In addition, DNA seemed too simple to be the genetic material; it contains only four different kinds of building blocks (bases, nucleotides), and it had not been generally recognized as a macromolecule.

Avery's discovery not only established the chemical nature of the gene: It also revealed the possibility of transferring genes between bacteria. Within 4 years Lederberg, inspired by this finding, discovered two additional mechanisms: *conjugation* (transfer through a bridge between two bacterial cells) and *transduction* (transfer by a virus). Their evolutionary significance is profound. For while genetic variety arises ultimately from mutations, it would develop very slowly if mutations could accumulate only within a direct line of descent. Hence it is not surprising that the capacity for at least occasional gene transfer should have evolved already in the prokaryotes (that is, bacteria, which lack an organized nucleus and multiply asexually), even before the regular reassortment of genes, in sexual reproduction, emerged in the eukaryotes (organisms with a membrane-enclosed nucleus in their cells).

Now that the simplest cells could be used for study of genetic processes common to all organisms, their former disadvantages turned out to be advantages. Because of the small size of bacteria and their rapid doubling, 10^9 organisms could be grown from a single progenitor overnight in a test tube. Moreover, unlike the classical geneticists, observing at most a few thousand progeny, a bacterial geneticist could select for a particular recombinant or mutant among 10^9 cells. Bacteria thus became the organisms of choice in the pioneering studies in molecular genetics, illustrating Pasteur's dictum that "the secrets of the infinite will be found in the infinitely small."

Bacterial viruses (phages) also played a large role. Not only are they much simpler than bacteria, but the characteristic mechanism of viral multiplication that they revealed (release and translation of their nucleic acid in the host cell, followed by reassembly) provided additional advantages for study. Virology has grown into a major discipline, with growing implications for evolution and for cell biology, as well as implications for infectious disease.

DNA Structure: The Many Uses of the Complementary Strands

Perhaps the main reason for the delayed response to the genetic role of DNA was that this fact by itself failed to illuminate any problems in genetics. The situation changed dramatically in 1953 when Watson and Crick proposed a double helical structure for DNA, since the complementarity of the two strands immediately answered the old question of how genes can code for their own replication. The unusual style of this discovery launched a tradition, in the emerging field of molecular biology, that emphasized a sharp focus on key problems, creation of bold models, and intense discussion within a network of communicants.

Molecular genetics now rapidly grew through interaction of a succession of new concepts and new methods, much like the linked evolution of man's brain and his hands. The results have provided deep insights into many aspects of DNA structure and function. Today we know much about how genes are replicated enzymatically, maintain extraordinary fidelity, occasionally mutate, are transcribed into RNA, are expressed as protein sequences via a genetic code, are regulated in their expression, recombine in new arrangements, exchange between a virus and a cell chromosome, and expand in number as more complex organisms evolve.

In short, the structure of DNA provides for biology, as the structure of the atom does for chemistry, a set of principles and properties that account for an enormous variety of phenomena, and for the emergence of an enormous variety of arrangements of matter. Studies in molecular genetics have also filled in the missing elements in Darwin's theory of evolution, providing a detailed physical basis for heredity and for its variation. In addition, molecular genetics has provided the most direct support for the Darwinian picture: divergence in DNA sequence among species shows the predicted parallel to evolutionary distance, and the irreversible information transfer from DNA to protein ensures that the environment cannot direct a Lamarckian change in the genetic message but can only select. Accordingly, the implications for human origins are inescapable. Except for those skeptics who are willing to discard rationality, Darwin's theory has now become Darwin's law.

The study of DNA also opened a new world of macromolecular chemistry. Although ordinary methods of preparation yielded fragments with an average mo-

lecular weight of a few million, very gentle lysis could yield much larger intact DNA chains, from viruses and even from bacteria (Fig. 1). Moreover, when the hydrogen bonds between the strands are weakened by high temperatures, the strands separate (denaturation); and at an intermediate "annealing" temperature they can pair again. By measuring the extent of pairing between two kinds of DNA (or DNA and RNA) the degree of similarity in their sequences can be determined. This in vitro hybridization has provided a major technology used for measuring evolutionary distances, identifying specific sequences, directly mapping genes, and studying gene expression and regulation.

An interesting further property of DNA is that A · T pairs (which have two hydrogen bonds between adenine and thymine) separate more easily than G · C pairs (which have three between guanine and cytosine). Nature puts this property to good use. Regions rich in A · T are found at sites where a double strand must separate (for example, DNA-DNA at sites of initiation of transcription of a strand into complementary RNA, and RNA-DNA at termination sites where the transcript must be released). Moreover, complementary sequences within a strand of DNA or RNA permit it to fold into a hairpin loop, which can play a role in regulation; and the stability of the loop is influenced by the ratio of the G · C to A · T pairs in its double-stranded stem.

Fine-Structure Genetics: Recombination, Mutations, and Repair

Even before analysis of the sequence of bases in DNA became feasible the ingenious use of purely genetic methods in bacteria defined the gene in molecular terms.

Classically the gene was defined both as a unit of function (recognized by the phenotypic effect of a mutation) and as a unit of recombination (identified by its location in a chromosome); genetic mapping depended on the assumption that the frequency of recombination between any two loci on the same chromosome is proportional to the distance between them. The gene was thought to have some definite molecular structure within the chromosome, recombinations occurring at special intervening linker regions. A radically different picture emerged, however, when Benzer found it possible to increase the resolving power of recombinational analysis a millionfold by selecting wild-type recombinants (as rare as 1 in 10^9) between independent mutations in a particular locus. The lower limit of recombination distances was thus shown to be adjacent bases: recombination can occur at any site in the DNA.

This technique further showed that the end of one phage gene and the beginning of an adjacent one were also adjacent nucleotides. There are thus no molecular discontinuities in the chromosome; functional units are created only in the reading of the sequence of bases (like the reading of a computer tape), and not by any other structural differences. Moreover, a gene could be functionally defined as the DNA sequence coding for a polypeptide: some protein enzymes contain only one kind of polypeptide, but others are aggregates of two kinds and hence are coded for by two genes.

Benzer's studies also shed light on the mechanisms of mutagenesis. Although mutations are random over large chromosomal distances, fine-structure mapping showed that their frequency varies widely from one base to another, over as much as a 500-fold range. Moreover, spontaneous mutations were found to have a very different distribution of sites from the additional mutations induced by incorporating synthetic analogs of bases, which pair less accurately than natural bases. Subsequent studies showed that most spontaneous mutations do not arise, as had been assumed, by mispairing, but are due to misalignment of strands during replication or during recombination (that is, frameshift by insertion of an extra base or by omission of a base).

The double-strandedness of DNA is used in nature not only in replication and in recombination (in which first one and then the other strand is broken and rejoined), but also in repair of replication errors (and of radiation damage). Repair was perhaps the most unpredicted property of DNA, but in retrospect it can be recognized as a necessity to account for the extraordinary stability of DNA, and for its extraordinary fidelity of replication (6×10^9 base pairs in each of our cells replicating accurately generation after generation). In the major repair mechanisms one of the two strands is cut, and a short length (containing an error) is excised; the other strand preserves the continuity of the whole, and it also provides a template for replacing the missing segment.

Enzymological studies, starting with the formation of DNA in vitro by Kornberg, have shed much light on the processes of replication, recombination, and repair; some of the same enzymes are shared in all three processes. The mechanisms that regulate the initiation of DNA replication, thereby regulating cell division, are still not known. The biochemical studies on DNA damage, mutagenesis, and repair have converted much of radiation biology from a descriptive study to a growing branch of biochemistry.

While classical genetic recombination occurs only at regions of extensive homology, a quite different, site-specific mechanism of recombination has been found to insert certain kinds of nonhomologous DNA into a chain. This mechanism was discovered with certain phages, whose DNA is occasionally integrated into a specific site in the host chromosome by an enzyme that recognizes specific (but not homologous) sequences in the two chains of DNA. A variety of other specific insertion sequences (IS), recognized by cognate enzymes, have now been identified, especially in plasmids, which are autonomously replicating small circles of DNA (see Fig. 1), closely resembling viruses, but not lethal to the host and not coding for a viral coat. A very active field today is concerned with the mechanisms of transfer of these movable genes, and with their role in gene regulation and in the exchange of genes between chromosomes, plasmids, and viruses.

Thus variations in DNA sequence are used not only to code for different products but also to influence other properties, such as mutability and transfer; another major use, in gene regulation, is discussed below. We have no doubt not yet seen the end of the series of tricks that evolution has been able to play with the four cards in the DNA deck.

Gene Expression and the Genetic Code

Once it became certain that a sequence of bases can specify the sequence of amino acids in a corresponding polypeptide chain, the code connecting the sequences and the mechanism of translation became major research objectives. In Zamecnik's group, which first achieved protein synthesis in cell extracts (that is, incorporation of radioactively labeled amino acids into large polypeptides), Hoagland showed that the system required not only amino acids, ribosomes (large, sedimentable particles containing protein and RNA), and enzymes but also a mixture of transfer RNA (tRNA) molecules (of about 80 nucleotides), and enzymes

that could covalently link each to a particular amino acid. The tRNA thus serves as an adapter between a coding unit on the ribosome and the corresponding amino acid.

Though the in vitro system worked, straightforward biochemical analysis failed to reveal a major component: a special, rapidly turning over form of RNA, messenger RNA (mRNA). This material was recognized by Jacob and Monod, on the basis of kinetic studies of gene regulation (see below). Thus when the gene for the enzyme β-galactosidase is turned on or off, the production of the protein responds fully within 2 to 3 minutes. Ribosomes are much too stable to account for these kinetics, and therefore it was postulated that the gene forms a labile RNA transcript (with a half-life of only 2 or 3 minutes), and that this messenger attaches to ribosomes and directs the polypeptide sequence that they form. As a result of this prediction, the labile mRNA (and its sequence homology to DNA) was soon directly demonstrated. Moreover, the enzymatic mechanism for RNA polymerization (transcription) on DNA has now been worked out in considerable detail.

Meanwhile, the code was unexpectedly cracked open by a biochemical approach, when Nirenberg and Matthaei added a synthetic homopolymer of ribonucleotides, poly(U) (polyuridylic acid), to their test system. The resulting synthesis of polyphenylalanine pointed to UUU (U, uracil) as the codon for phenylalanine. With this breakthrough, what had long seemed a distant holy grail was soon reached by testing various synthetic polynucleotides, and within 3 years the complete code was identified, including signals for start and stop.

The continuous reading of trinucleotide codons explains why frameshift mutations lead to gibberish (or to premature termination) in the distal part of a gene. However, in some viruses, where economy of size is evidently of paramount value, the same DNA sequence is used to code for more than one protein through initiation of translation in more than one reading frame.

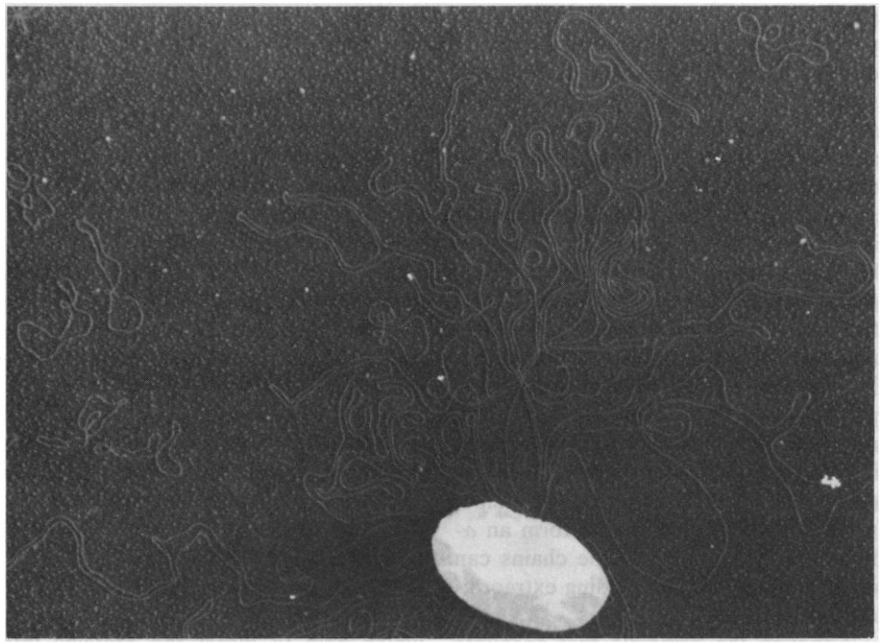

Fig. 1. A lysing bacterial cell, releasing loops of chromosomal DNA and also multiple copies of a plasmid (an autonomously replicating, smaller circle of DNA). [Courtesy of H. Potter and D. Dressler, Harvard University]

The Ribosome and Protein Synthesis

The machinery of protein synthesis is elaborate, but the individual steps are, in principle, simple. As the ribosome moves along the messenger, a cycle of steps at each new codon adds an amino acid to the end of the growing chain, which may reach a length of several hundred residues before release at a termination codon. In initiating and in terminating translation at the correct sites in mRNA, the ribosome cooperates with special protein factors.

The ribosome, containing three large RNA molecules and 52 different protein molecules, is an extraordinarily complex molecular aggregate. Nomura's in vitro reconstitution of active ribosomes from their separated constituents, by spontaneous assembly under appropriate conditions, has been a triumph of biochemical manipulation. The intricately intertwined topological relations of the various components of the ribosome, and the binding sites for the many ligands that go on and off at successive steps in its cycles, are a major challenge now being studied by chemical, physical, immunological, and electron microscopic techniques. An even greater challenge for the future is the definition of the many conformations through which the ribosome must cycle.

The ribosome is not simply a passive bench on which mRNA moves and is translated. It uses energy to ensure accurate and firm binding, in the proper order, of several ligands; it provides an "editing" process that uses energy to reject the incorrect aminoacyl-tRNA's that are occasionally bound; and certain ribosomal mutations, as well as antibiotics (such as streptomycin), can alter the fidelity (accuracy) of translation.

Protein synthesis requires an extraordinary amount of energy: more than four energy-rich bonds from ATP per peptide bond formed, whereas the same peptide bond costs only one energy-rich bond in enzymatic, nonribosomal syntheses of small peptides. The high price is evidently paid to gain several advantages, including high fidelity, secure retention of the growing polypeptide chain, rapid chain growth (about 15 amino acids per second), and above all flexibility. The mechanism can produce any length of chain, and any sequence, that has evolutionary survival value.

One Dimension into Three: Protein Structure

A major advance in classical biochemistry was the finding that the major working machinery of a cell consists of hundreds of different proteins, each with one or more sites that combine noncovalently with specific ligands. A virtually infinite variety of specific combining sites are thus found—in enzymes, antibodies, antigens, regulatory proteins, membrane transport proteins, and the structural proteins that help to hold cells and organs together. To account for these specific sites on the surface it is necessary to know not only how one-dimensional information is translated from a nucleic acid sequence into a polypeptide sequence, but also how such information can create specific three-dimensional shapes.

The solution that emerged is very

simple. The one-dimensional polypeptide product of a gene folds spontaneously into its final three-dimensional shape. The chain thus contains in itself all the needed information, in the form of attractions and repulsions between the side chains of the 20 different kinds of amino acid residues. These interactions involve positive and negative charges, hydrogen bonds, the attraction of hydrophilic residues for aqueous or polar surfaces, and the attraction of hydrophobic residues for each other (or for lipids). Protein shapes, like nucleic acid strand pairing, thus depend on multiple weak noncovalent bonds. But while the bases in DNA pair only at precisely fixed angles, yielding little flexibility of shape, protein chains not only can form an α-helix (Pauling), but the side chains can interact at any angle, yielding extraordinary plasticity.

Although the principle that accounts for three-dimensional specificity is as fundamental as the principle of storage of information in DNA, it did not have so dramatic an impact, for the evidence accumulated gradually, starting with Anfinsen's finding that a small enzyme, ribonuclease, could regain some activity if annealed after denaturation. The subsequent synthesis of many active enzymes in vitro showed that even large polypeptides will spontaneously reach an active form if allowed to fold as they emerge from the ribosome—quite a different process from trying to refold correctly after multiple denatured chains have tangled together in a heated solution (such as a boiled egg).

Several techniques have been of great importance in detecting and in isolating specific proteins, and in analyzing their detailed molecular structure. Chromatographic methods (based on size, charge, specific binding affinities, and other features) can easily resolve and isolate hundreds of proteins in the mixture from a cell. Moreover, specific proteins can be identified with extraordinary sensitivity by interaction with antibodies. Determination of the sequence of amino acid residues, first accomplished by Sanger, is now made easy by automatic sequenators. X-ray crystallography, extended to proteins by Perutz, has reached a power of resolution that makes it possible to identify in three dimensions the spatial relations of all the atoms of a crystalline protein. A major challenge is to learn how to predict from a polypeptide sequence which of the large number of possible conformations will have the lowest free energy level, and hence will represent the natural state. Finally, enzymology has moved to new levels, starting with Phillips' use of x-ray crystallography to demonstrate a binding site that closely fits the substrate. Affinity labeling, with reactive analogs of substrates, is also used to study the relations between substrate and the residues making up the combining site.

Studies of protein structure have further shown that the products of many genes do not remain as such but serve as precursors, which undergo various secondary, posttranslational modifications, including for example, cleavage of a few residues at one end, or attachment of phosphate, carbohydrate, or many other groups at various positions. A rapidly expanding area today is concerned with the role of these reactions, including both reversible modifications, which regulate the functional activity of various proteins, and irreversible modifications, which help to direct the proteins into specific locations (such as in membranes or within organelles of higher cells).

Regulation and Allostery

Regulatory relations between organs have long been a major topic in physiology. The more recent elucidation of the metabolic pathways in a bacterial cell led to the discovery of an equally elaborate system of intracellular controls. Two general sets are found, namely, induction (or repression) of the activity of specific genes, and inhibition of the activity of specific enzymes. For example, addition of a given amino acid represses further formation of the enzymes of the corresponding pathway (which are gradually diluted out during further growth), thus sparing the synthesis of unnecessary RNA's and proteins. At the same time, the added amino acid immediately spares its own unnecessary endogenous synthesis by directly inhibiting the first enzyme of its pathway. Both these mechanisms were discovered through their effects on the foreign economy of the cell, but they are equally important in feedback loops that regulate its domestic economy. Thus an endogenously formed end product is normally kept at a constant intracellular level in steady-state growth through the effect of that level on the activity, and on the formation, of enzymes of its pathway. Similar feedback must regulate the formation of all cell components, large or small. For example, synthesis of ribosomal components is repressed by an elaborate mechanism that is activated by limitation in the supply of any of the 20 amino acids: translating ribosomes are held up by decreased aminoacylation of any species of tRNA.

Induction of the formation of an enzyme, by its substrate, was the first regulatory mechanism to be basically understood. Work of Jacob, Monod, and Pardee on mutants altered in this process revealed the operon, in which a repressor protein, binding to a controlling locus (the operator), prevents the adjacent region (promoter) from binding RNA polymerase and hence initiating transcription of an adjacent sequence of functionally related genes. Whether or not the repressor is active (that is, can bind to the operator) depends on whether it is free or is complexed with a specific small molecule (inducer). The protein must therefore recognize both a specific DNA sequence and a specific controlling small molecule.

Many other mechanisms of gene regulation have now been discovered in bacteria—for example, regulatory proteins that activate rather than repress operons; and, very recently, a coupling between transcription and translation that prematurely terminates formation of a messenger when the presence of adequate end product indicates that it is not needed. Some agents [for example, cyclic adenosine monophosphate (AMP)] are pleiotropic, influencing a whole class of operons with parallel functions, rather than a single operon. This class is of particular interest as models for the presumed cascades of gene regulation in differentiation in higher organisms.

Current work is concerned with analyzing in detail how regulatory proteins bind to the DNA of the operator, by recognizing aspects of the bases that are accessible to the outside without strand separation. Such work was initiated when repressor proteins were ingeniously isolated by Gilbert and by Ptashne, despite the presence normally of less than ten molecules per cell.

The second major class of regulatory mechanisms acts not on genes but on enzymes. It was known that mutants blocked in biosynthesis do not accumulate the substrate of the blocked reaction until they have exhausted the supply of required end product. Umbarger and Pardee then each found that the end product directly inhibits the first enzyme of its pathway. Pardee showed, in addition, that these regulatory enzymes can bind two ligands of different shape in different sites (allostery). These sites interact conformationally (Monod and Koshland), the inhibitor shifting the equilibrium toward an inactive conformation of

the enzyme, and the substrate shifting it toward an active conformation.

The kinetics of allosteric enzymes exhibits an additional property of great value for the organism: the sensitivity to small shifts in concentration is increased by the presence of multiple subunits that interact conformationally, so that the binding of the first molecule of substrate (or of end product) increases the affinity of the enzyme for additional molecules. The resemblance to the sigmoid curve for the binding of oxygen by hemoglobin is an example of the increasing overlap of studies in various areas of biology; Perutz's work on the conformational effect of oxygenation on hemoglobin now provides the best model for the similar cooperative changes between subunits in regulatory proteins.

The ability of certain proteins to snap back and forth between two conformations, with different activities, has a significance far beyond regulation of enzyme action. As Monod emphasized, it is also the key to the action of operon repressors, and it undoubtedly plays a role in many other phenomena, including responses of cells to hormones, drugs, and neurotransmitters; responses of sense organs to stimuli; membrane transport and nerve conduction; interactions of antibodies with cells; muscle contraction and cell and organelle motility; and phagocytosis.

Allosteric proteins thus represent a second mechanism for the molecular transfer of information. They do not provide blueprints for molecular patterns, as do nucleic acids, but they sense concentrations of compounds, thus serving to integrate metabolism within an organism and also to mediate the interaction of its genes with its environment. In addition, their shifts in conformation, like the smaller shifts in the active site of an enzyme interacting with its substrate, emphasize that time is a significant dimension in protein structure.

Gene Manipulation: Recombinant DNA

The extension of molecular genetics to higher organisms has recently been greatly advanced by the development of a methodology in which short segments of DNA from any source can be spliced into a circular DNA vector (plasmid or bacteriophage) that can be grown in bacteria (cloning). These techniques have been extensively reviewed.

It is now a relatively simple matter, by "shotgun" hybridization of the total DNA of a mammal, to obtain a mixture of about 10^5 bacteria that collectively contain the whole genome. Those containing a specific spliced segment can often be identified by their hybridization with a radioactive mRNA, or by their translation into identifiable proteins. Moreover, by use of several restriction enzymes, which each cleave DNA at a specific short base sequence, the segments can be further cleaved into definite fragments short enough (less than 200 nucleotides) to be sequenced by simple techniques developed by Maxam and Gilbert and by Sanger. (Incidentally, it seems likely that the volume of information acquired in this way will soon exceed the capacity of scientific journals.) The recombinant technique has already revealed major surprises about the organization of mammalian genomes. A major hope is that it will help to work out the elusive and complex mechanisms of gene regulation involved in the ontogeny of higher organisms.

The medical and commercial promise of the recombinant DNA technology (for example, for producing valuable mammalian protein hormones, antibodies, and interferon; for producing antigens for use in immunization; and for introducing nitrogen fixation in higher plants) has justifiably received wide attention, although so far only mammalian proteins have been obtained and those in very small yield. It seems reasonable to expect rapid improvement in yields, and extension to innumerable additional products. Since gene regulation in eukaryotes is quite different from that in prokaryotes it seems possible that the cloning of recombinant molecules in the simplest eukaryotes, the one-celled yeasts, will be of great value, both for fundamental studies and for commercial production. Cloning in these organisms, and in cultured animal cells, is rapidly expanding.

Membranes

Virtually all natural membranes have a similar basic structure: a bilayer of lipids (with polar groups at the surfaces), providing a two-dimensional fluid matrix in which proteins are embedded and can move laterally. The resulting osmotic barrier retains even very small molecules (and mineral ions) within the cell, their entry and exit being controlled by specific transport systems traversing the membrane.

Investigations in lipid chemistry have long been limited by difficulty in obtaining pure compounds, and also in studying their reactions in an aqueous environment. However, in recent years gas liquid chromatography lent precision to the analysis of lipids. In addition, electron microscopy greatly increased interest in membranes; solubilization with detergents made it possible to identify and characterize the many proteins in natural membranes; methods of labeling either side of the membrane, and of splitting membranes by freeze-fracture along natural lines of cleavage, made it possible to localize these proteins; and spin-labeled probes shed light on the mobility of molecules within the membrane. Artificial vesicles (liposomes) can be constructed, incorporating desired mixtures of proteins and lipids in the membrane and containing desired solutes within the lumen. With these new methods the study of membranes is thriving. Moreover, one can foresee the possibility of reconstituting a self-replicating cell.

Some transport systems simply facilitate transport in either direction, while others serve as pumps, building up a high intracellular concentration of specific permeants from a dilute extracellular source. Like genes, these pumps were discovered belatedly in bacteria, which were long assumed to have a simple semipermeable membrane. However, they may have evolved early, since the early organisms probably had to assimilate very dilute nutrients.

Despite intensive study, including the isolation of many transport proteins and their reconstitution in active vesicles, the detailed mechanism of active transport has remained refractory to direct biochemical analysis, because transport systems lose their characteristic vectorial property when solubilized. In a plausible mechanism, a transport protein would have a binding site able to face either side of the membrane, and it could use metabolic energy to lower the affinity of that site for the permeant (thus promoting unloading) when facing the inside.

A dramatic advance has been Mitchell's recognition that the vectorial orientation of the membrane, and its insulation against nonspecific flow of molecules (and protons and electrons), are essential for deriving energy from the metabolic flow of electrons (reducing equivalents) from substrates to oxygen. This electron transport, through a sequence of proteins embedded in the membrane, is accompanied by extrusion of protons (H^+) from the cytoplasm to the outer surface. The resulting electrochemical gradient (protonmotive force) is the source of the energy used for build-

ing up ATP, for active transport, and, in bacteria, for cell movement by the rotation of helical flagella.

Plants form ATP by a related mechanism, through a proton difference (across a membrane) created by radiant energy captured by chlorophyll. The mechanism is being explored in detail, and practical applications are anticipated. Since all life depends on the continual conversion of CO_2 to organic matter, by the use of both reducing power and ATP provided by photosynthesis, the mechanism is of wide interest.

The secretion of proteins across membranes has become a particularly lively field, with the finding that many secreted proteins are formed as a precursor, with an initial, predominantly hydrophobic "signal peptide" sequence that directs entry into the membrane and later is cleaved. The mechanism of continued penetration of the chain across the membrane is still obscure.

Signal peptides are also found in many proteins that are incorporated into membranes. The specificity of membrane morphogenesis is a challenging area, for various kinds of membranes in a cell are highly differentiated in composition, even though many are continuous with each other and their proteins can move in the lipid matrix. In addition, the two sides of a membrane differ markedly in lipid composition. Hence, even though earlier studies of lipids, in aqueous solutions, emphasized their nonspecific aggregations, the elucidation of specific intramembranous associations is now a major challenge. Although membrane proteins can now be solubilized and isolated, the study of their detailed structures lags far behind that of water-soluble proteins.

Eukaryotic Cell Biology

While molecular biology was being developed largely in bacteria, electron microscopy was revealing a wealth of previously unsuspected organization in animal and plant cells, and the vague term "protoplasm" was replaced by recognition of a variety of membranes, filaments, and organelles. Modern cell biology arose as such morphological studies, begun by Palade and Porter, were integrated increasingly with studies of function. The techniques used included ultracentrifugal separation of cell fractions, selective staining of specific proteins with antibodies carrying fluorescent or other labels, and autoradiographic localization of macromolecule synthesis; in addition, the genetic and molecular principles and techniques emerging from work on bacteria have been increasingly assimilated. Huxley's studies on the alternating parallel filaments of actin and myosin in skeletal muscle were perhaps the first great success in understanding cell organization and assembly, and their insights into the mechanism of contraction provided the model for present studies on the localized contractions involved in such cellular activities as motility, changes in shape, and mitosis.

In another direction, in vitro cultivation of the slow-growing fully differentiated cells of animals or plants has been facilitated by various technical developments, including suppression of bacterial growth by antibiotics, and improved understanding of nutritional requirements and cell adhesion. Such cultured somatic cells not only can be used for study of cell function and its regulation, but they can replace whole organisms in many genetic studies, including mutagenesis, mutant selection, recombination, and gene mapping. I shall review the cell biology of eukaryotes all too briefly, noting only some major differences from prokaryotes.

The mechanisms of gene replication and expression are basically similar in the two groups, but with substantial differences. In the multiple, larger chromosomes of eukaryotic cells the DNA is organized into an elaborate, regular pattern of units called nucleosomes. These are formed by coiling the DNA around a set of basic proteins (histones), while a wide variety of other proteins are responsible for the selective, stable repression of a large fraction of genes (perhaps 90 percent) in each differentiated cell type. In addition, in higher eukaryotes a large fraction of the DNA is repetitive and is not transcribed. The function of this DNA is a major challenge: one possibility is that it has no function for the cell but is merely a by-product of the capacity of the "selfish" DNA to promote its replication even when it does not affect the phenotype. Finally, mitochondria and chloroplasts carry autonomous blocks of DNA as well as characteristic ribosomes, and it seems likely that these intracellular organelles are descended from bacteria and algae that parasitized the early eukaryotes.

The mRNA also exhibits a more complex behavior in cells with a membrane-enclosed nucleus. The immediate RNA transcript, formed in the nucleus, is much larger than the final messenger. It is processed in the nucleus, by elimina-

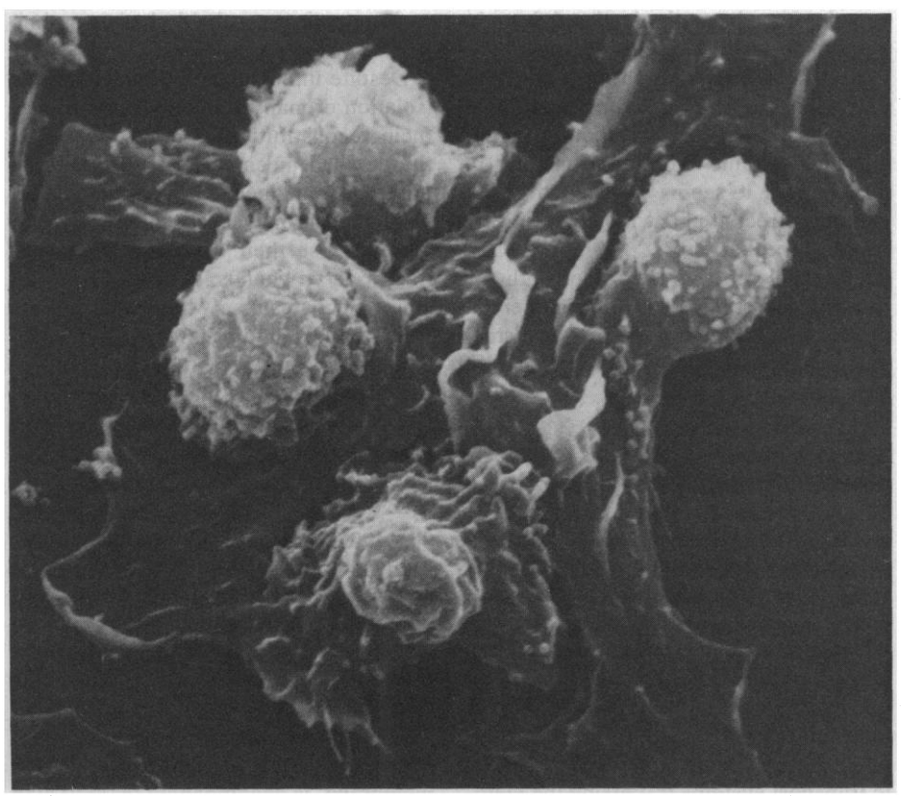

Fig. 2. Scanning electron micrograph of four lymphocytes (spherical cells) transiently attached to a large mononuclear phagocyte (macrophage). This cellular interaction is a necessary step in activating the potentiality of a lymphocyte to secrete a specific antibody. The molecular basis for the activation is unknown. [Courtesy of K. Ziegler, R. Cotran, and E. Unanue, Harvard Medical School]

tion of large untranslated segments and also by additions to the ends. The resulting relatively stable mRNA molecules then join the ribosomes in the cytoplasm. In addition—and this was a great surprise—the DNA sequence for a given protein is not found as a single contiguous series of bases, as in bacteria. Instead, several regions of a gene, each coding for part of a polypeptide chain, are separated by untranslated intervening sequences. The messenger is thus not a direct transcript of the DNA: it is formed from the transcript by special enzymes that cut out and splice together the proper segments. The full significance of this splicing remains to be discovered. It probably plays a regulatory role, and it may also increase the variety of products of the genetic information by allowing RNA from different regions of the genome to recombine.

On the whole, eukaryotic gene regulation is little understood. In the hope that its principles may be most easily discovered in the simplest eukaryotes, the genetics of yeast has been expanding rapidly.

Bacterial and animal cells differ also in the nature of their surface envelope. In bacteria the membrane is surrounded by a protective, rigid external wall, while in animal cells the plasticity of the cell envelope makes possible an enormous variety of shapes, as well as rapid changes in shape. For example, the differentiating cells of an embryo change shape as they move into their positions, as do scavenging cells (phagocytes) in crawling into tissues and taking up particles. The scanning electron microscope has revealed beautiful, unexpectedly complex patterns in the surface protrusions and the contacts of many cells (Fig. 2). In addition, communication between adjacent cells in animals involves several kinds of specialized junctions between membranes.

A major development in the past decade has been the discovery of the cytoskeleton. One of its elements is a set of relatively rigid struts, called microtubules (Fig. 3). Another is a set of several kinds of microfilaments, two of which (actin and myosin) are also major constituents of muscle. Receptors for the attachment of these fibers to the inner surface of the cell membrane are being identified. Microtubules can be rapidly elongated in one region and removed in another by aggregation or disaggregation of their constituent protein molecules. Variations in their location and that of the filaments, and movement of the latter, account for cell movement and changes in shape.

Study of the dynamics of the cytoskeleton, and of its response to stimuli, is advancing rapidly. In muscle the sliding of actin relative to myosin filaments has been traced to transient cross-bridges between them, some form of molecular contraction leading to exchange of one cross-bridge for another. The mitotic apparatus also contains myosin (and microtubules), but the mechanism of its orderly movement of chromosomes remains a problem.

At another level, studies on the specific receptors for various hormones and drugs, located on the surface membranes of animal cells, are revolutionizing endocrinology and pharmacology. For example, epinephrine action on the surface of target cells results in the intracellular formation of a regulator (cyclic AMP) that can influence many enzymatic reactions, usually by some amplification system (for example, activating an enzyme that phosphorylates other enzymes). Moreover, chemical signals between organisms (particularly in lower mammals and in insects), via species-specific and strain-specific compounds (pheromones), play a large role in communication. These molecules can convey information even at exceedingly low concentrations. It is clear that a great variety of such interactions with surface receptors await exploration.

Developmental Biology, Immunology, and Cancer

In contemporary biology the most challenging area at a molecular level is surely developmental biology. But the advance of molecular biology, like the earlier advance of classical genetics, has been blunted by this challenge. Whereas the genetic program is expressed very directly in the specification of a polypeptide sequence, and slightly less directly in protein folding, in development the contribution of each gene is interwoven with that of innumerable others. In addition, each gene is not simply responding reversibly to immediate changes in its surroundings, as in metabolic regulation: an added dimension is supplied by cumulative, persistent effects of the past history of the cell and of its neighbors. At present many informational molecules are being identified that induce specific developmental responses. However, with so many steps intervening we may not be able to trace the developmental program of the genome in terms of linear sequences of information transfer. Just as the understanding of protein function required the novel concepts of spontaneous folding and allosteric shifts, so a new conceptual framework may be necessary to explain how gradients of regulatory molecules

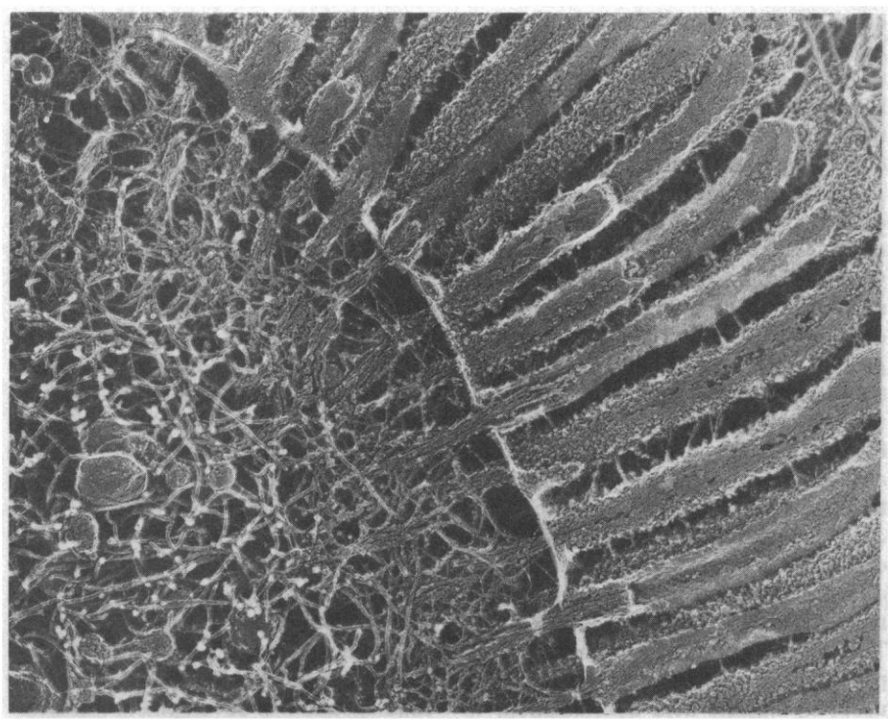

Fig. 3. The cytoskeleton within an intestinal epithelial cell. Bundles of actin filaments enter the villi (parallel cylindrical protrusions at the surface facing the lumen of the intestine, toward the right). Other kinds of filaments, and roughly spherical organelles, are seen deeper in the cell (lower left corner). Prepared by quick-freezing, fracturing, deep-etching, and rotary shadowing with platinum. Original magnification, × 80,000. [Courtesy of N. Hirokawa and J. Heuser, University of California Medical School, San Francisco]

and even of physical forces, mediating reciprocal interactions between cells and changing with time, induce the formation of a sequence of novel patterns.

Under these circumstances we can ask many questions, in molecular terms, to which the answers are very incomplete or altogether absent. For example, how are the proper sets of genes turned on and off, presumably in large groups, in each kind of differentiated cell? How do such cells perpetuate their selective gene expression, by a kind of positive feedback? Why is the differentiation of animal cells largely irreversible, while plant cells can dedifferentiate and give rise to a whole organism? What kinds of reciprocal communication between neighboring cells trigger their succession of changes in a differentiating embryo? How many different stages of selective gene expression does a cell pass through before reaching full differentiation? In embryogenesis, what directs the differentiating cells of the early embryo to migrate into the proper positions, and what specific interactions between cell surface molecules stably organize cells into an organ? By what subtle mechanisms can genes dictate this morphogenesis with such extraordinary specificity that it yields, for example, not only a face, but one with a strong parental resemblance? As the fertilized egg becomes, say, the 10^{14} cells of a human adult, or as a damaged organ regenerates, what signals tell each organ to cease growing when the proper functional mass and shape has been reached? Finally, what mechanisms are responsible for the aging and death that are an inevitable part of the cycle of sexual reproduction in higher organisms?

A number of promising new approaches have been developed. One of these observes the developmental effects of various mutations; but as I noted above, such single-gene effects may illuminate only limited angles of a highly polygenic process. The use of cultured tissues, to try to identify the chemical factors and the cell-cell interactions that guide differentiation, has already proven fruitful and will surely expand; many new factors are being identified. Cultured cells can also be fused, and such mixing of nuclei and cytoplasm in different states of differentiation is throwing light on the mechanisms that turn genes on or off. The recombinant DNA methodology promises to provide probes that can be used, by nucleic acid hybridization, to identify the active and the inactive genes, and also to recognize the gene amplification or deletion that is suspected to occur in some differentiated cells. In another valuable approach, chimeric mice can be formed by mixing separated cells of an early embryo with cells of a genetically different embryo (or with mutant cells selected in vitro), followed by reimplantation in a host uterus.

Immunology, long a ward of microbiology, has become a major, independent discipline. It has been widely regarded as a model for differentiation, since it involves the production of perhaps a hundred thousand cell lines in an organism, each yielding a different specific antibody. Studies on sequence have revealed a constant region characteristic of each of several major classes of immunoglobulins, and a set of variable regions responsible for the specific binding sites. To form the latter, it seems likely that at a certain stage of development cells of the immune system have a very high rate of mutation, and perhaps also of recombination, in a localized genetic region.

Knowledge of the cellular aspects of immunology is advancing rapidly, including the complex interactions of several kinds of cells in the immune response (Fig. 2), and an intriguing involvement of the major "transplantation" antigens of the cell surface in these interactions. Because the products of the immune system can be detected with great sensitivity and specificity, studies with this system may well reveal general cell-cell interactions that are less analyzable in other systems.

The extraordinary specificity of antibodies has also made them extremely valuable tools for the recognition, quantitation, and isolation of individual proteins: few cross-reactions are seen between thousands of unrelated proteins despite the inevitable sharing of short sequences of amino acids. However, the antibodies elicited in an animal in response to any antigen are not pure but are a mixture of molecular species, differing in the site on the antigen that they bind, and in their affinities. Recently Milstein, fusing antibody-producing cells with tumor cells, has obtained cell lines (hybridomas) that produce a single molecular species of antibody and that can be cultured indefinitely (unlike normal cultured mammalian cells). These "monoclonal" antibodies promise to revolutionize the study of the immune response and the medical and analytical uses of its products.

In recent years, cancer research has received increased public attention and funding, and this development has clearly contributed to the rapid expansion of animal cell biology. In the cancer field itself substantial progress has been made in characterizing cancer cells, in analyzing the action of tumor-producing viruses, and in relating carcinogenesis to mutagenesis. However, despite enormous effort most of the basic questions remain unanswered. What mutations are responsible for the carcinogenic effect of most mutagens? What products of viral genomes, or of their interaction with host genes, are responsible for the altered growth properties of cultured cells "transformed" into cancer cells by oncogenic viruses? How do alterations in differentiation contribute to carcinogenesis, and can they do so even without a genetic change? Why do cancer cells cease to respond to the mechanisms that control the growth of normal cells? What changes in surface properties permit cancer cells to leave an organized tissue and set up colonies (metastases) elsewhere in the body? Can the antigens of cancer cells provide a basis for immunotherapy? How do DNA repair mechanisms affect the response to various doses of radiation or to mutagenic chemicals? How do genes influence susceptibility to various kinds of cancer? Advances in the understanding of normal cell biology, and in the techniques for its study, are essential for answering many of these questions.

Neurobiology

Neurobiology has emerged from the integration of electrical measurements of nerve cell activity with cytological, biochemical, and pharmacological tools, mostly applied to relatively simple animals (for example, leech and lobster). Its language and methodologies overlap increasingly with those of other areas of biology (including particularly endocrinology, concerned with another major set of mechanisms for conveying information within a higher organism). For example, certain potent poisons (such as botulinum toxin and bungarotoxin) have proved to be sharp tools because they act on specific steps in the formation, release, binding, or enzymatic destruction of a particular transmitter. The mechanism of action of nerve cells can thus be approached today as effectively as many other problems in cell biology.

The function of the brain, however, depends primarily on its wiring diagram, whose incredibly complex organization presents a totally different problem: the human brain has more than 10^{10} nerve cells, each connected on the average with perhaps 10^3 others. Studies in classical neuroanatomy have revealed in a rough way the grouping of cells of related function into regions, and the major tracts of fibers connecting these regions.

It is now possible, however, to study the organization of the brain more physiologically, and in much finer detail, by observing the effects of external stimuli on the activity of different cells and regions in the intact animal. In particular, earlier measurements could only average the activity of many cells, but microelectrodes can now detect the activity of a single cell—a shift analogous to improving the resolution of genetic recombination from the level of genes to that of nucleotides.

Another method depends on a radioactive analog of glucose to detect the increased metabolic activity of the regions that respond to a particular physiological stimulus: the analog is taken up as though it were glucose, but it cannot be metabolized, and therefore its radioactivity accumulates in the cells. Indeed, positron-emitting analogs can be used even in the intact human brain, to identify the regions responding to even verbal stimuli.

Studies on the visual system with these techniques have been particularly fruitful in approaching the problem of how external stimuli are integrated in the brain to yield perception of patterns. While cells in the retina respond to points of light, cells in the visual cortex have a more integrated response, each detecting a contrast between light and dark at a particular position and shape in the visual field. Moreover, the cells connected to corresponding parts of the retinas of the two eyes are arranged in an orderly way. The principles of organization that are emerging from this most accessible system will no doubt be pertinent to the much more complex integration that must occur in higher centers, to yield a thought or action or mood.

A basic property of the brain that now seems accessible to study at a molecular and cellular level is memory or learning. Studies with simpler organisms have indicated that the cellular mechanisms for learning (that is, for a persistent effect of an earlier stimulus) involve two kinds of alteration, some in the function of existing synapses (and hence in unknown ways in their molecular structure), and others in the formation of new synapses. But while rapid progress can be expected in identifying the detailed nature of these changes, the search for the physical basis for conscious learning is part of the infinitely more complex problem of consciousness itself—a set of phenomena that may simply disappear, like the smile of the Cheshire cat, as we try to dissect their causal connections. Bohr has suggested a strong analogy to the wave-particle complementarity of the electron—a paradox that turned out to be an essential part of the revolutionary expansion from classical mechanics to quantum mechanics in explaining the nature of matter. Perhaps consciousness will eventually be understood neurobiologically in terms of some as yet unknown concept—not a return to mind-body dualism, but a set of rules for intercellular information flow as novel as molecular information storage was a few decades ago.

Until very recently studies on brain function have been almost exclusively concerned with "tightly wired" cell-to-cell connections, although it was recognized that changes in the fluid environment of the cells—ionic, hormonal, or pharmacological—could simultaneously and selectively influence the activity of large groups of cells. A major new field has recently been opened up by the discovery of a variety of peptide neurohormones: compounds that can function like neurotransmitters, but that diffuse from their site of release far enough to influence many surrounding cells. This effect is probably important in the general alterations of response connected with mood, alertness and sleep, and various mental illnesses. Moreover, some neuropeptides (endorphins, enkephalins) are found to bind to the same receptors, in pleasure and pain centers, as opiates; they are evidently the physiological agents for which morphine is an analog, and increases in their level have been observed under conditions of stress that greatly diminish sensitivity to pain. New peptides are now being reported every few months—and other integrative mechanisms are no doubt waiting to be discovered.

If the function of the brain requires incredibly complicated patterns of interaction, the mechanisms that guide the development of the nervous system are even harder to imagine. On the other hand, the extraordinary variety and precision of specific locations in this system could turn out to be a virtue in analyzing the process of development. What kinds and number of chemical signals could possibly guide the billions of nerve fibers to their proper destination, and induce synapse formation there, in the growing embryo? Since the number of connections far exceeds the number of genes, it is clear that the connections must depend, like the other morphogenetic processes discussed above, on the sequential actions, in time and space, of the products of an unknown, large number of genes. Studies with intact animals have provided evidence that some synapses are set up provisionally and then withdrawn if not used, in a sort of Darwinian process. In the visual system, in particular, cortical cells that appear to be connected at birth become lost or nonfunctional if not used during a critical period early in life. Another approach uses cultured cells, where contacts of growing nerves with muscle are seen to induce synapse formation and electrical activity. Finally, a genetic approach to neural morphogenesis is now being pursued in nematodes and other lower animals, which have a nervous system small enough (a few hundred cells) so that every cell can be identified and its main connections worked out. Isogenic strains are useful in determining how much developmental noise (random variation) enters into the detailed patterns. Even more, various mutants provide valuable tools for perturbing development in well-defined ways, and for correlating altered structure with altered function. But as with other aspects of development, there must be many steps between individual genes and the processes that specify neural circuitry; hence we cannot expect genetic studies to lead directly to the algorithms, as they have done for the algorithms of protein synthesis.

Evolutionary Biology

Like the intracellular feedback of information that selects actual gene or enzyme activities from a program of wider possibilities, interactions of the individual organism (and of the social group) with the environment select for the multiplication of a particular genome (and therefore of its constituent genes) from a wider gene pool. As we have seen, molecular genetics has provided some valuable new approaches to the study of this evolutionary process. However, evolutionary biology is largely concerned with events at a very different level of organization, and its importance has been somewhat obscured by the triumph of molecular biology.

In a very cursory survey of recent developments, we might first note that the field has become increasingly mathematical—for example, in population genetics. In ecology many of the parameters have also become subject to quantitative study and have been amalgamated in mathematical models. Ecology has, in addition, aroused wide public interest, in reaction to the impact of technology on the environment.

Although classical and molecular genetics have both been built on the study of those qualitative phenotypic traits that

can be traced to a single gene, from an evolutionary (and a social) perspective the quantitatively variable, polygenic physical and behavioral traits are the most interesting ones in higher organisms. Their statistical analysis has yielded much information on gene-gene interactions and on gene-environment interactions, and it has been of great value in animal and plant breeding; but the ultimate goal of identifying the specific genetic pattern of a noninbred individual still seems very far off. The role of genes in human behavior in particular, although of great potential value for maximizing individual self-fulfillment, will probably be understood only imprecisely, and in general and statistical terms, until the genes involved can be directly identified in individuals. Meanwhile, discussions of the statistical evidence in this area have been clouded by polemics, as some environmentalists have leveled the charge of genetic determinism against those who conclude that both genes and environment play a significant role.

A major development has been the discovery of an enormous range of diversity within a species at the molecular level, as well as at the morphological and the behavioral levels; in populations of the fruit fly, or of humans, more than one-third of the measurable circulating enzymes are found in two or more different normal forms (polymorphism), in contrast to the earlier assumption of only a single wild-type form and rare mutants. Much of this molecular variation is not associated with any demonstrable selective value of one allele over another; indeed, at the level of DNA the polymorphisms sometimes involve synonymous codons, differing in a nucleotide but yielding the same protein. These findings have given rise to the neutralist hypothesis, which emphasizes the role of stochastic processes (rather than selection) in evolution. But selective advantages are often not obvious; and the search for cryptic selection pressures is an active field, combining molecular, physiological, and ecological studies.

Molecular genetics has led to several additional interesting conclusions or speculations about the evolutionary process. The recognition of movable genes, and the apparently high rate of localized mutagenesis in the creation of immunological specificity, both suggest mechanisms of variable genetic instability that may be of wide applicability. Moreover, viruses may turn out to have evolved primarily to transfer blocks of nucleic acid between organisms; and in the generation of plant tumors by incorporation of a bacterial plasmid we see that DNA transfer may occur even between very distant organisms. Accordingly, it is not inconceivable that all DNA in the living world may be part of an unbroken chain of low-frequency contacts. Finally, a general direction of evolution toward ever more complex organisms, possessing increased amounts of genetic information, is quite understandable. Duplication of a segment of DNA provides an organism with the basis for creating genetic novelty, without losing the original, valuable product, whereas deletion is much more likely to be harmful (or even lethal).

The most recent major development in evolutionary biology has been the focus on sociobiology: the search for the biological roots of social behavior in its various forms throughout the animal world. Much of the impetus for this development was resolution of the paradox of selection for altruism, in an inherently selfish system of selection. Two mechanisms have been recognized: kin selection (in which loss of an individual's genes enhances the multiplication of the same genes in relatives), and group selection (cooperation within a group favoring its survival in competition with other groups). As applied to understanding human nature, including both its universals and its diversity, the integration of sociobiology and neurobiology with the traditional approaches of the humanities and the social sciences will no doubt encounter resistance. Nevertheless, some advances, for example in determining the role of endogenous mood-determining neuropeptides, may soon become too relevant to ignore.

Conclusions

In the past two decades an extraordinary range of universal rules of cell organization have been revealed through the fusion of genetics and biochemistry and the development of sophisticated techniques for studying macromolecules. Molecular information transfer, outward from the DNA and inward from the surroundings, plays a central role. Another striking feature is the thoroughly mechanistic nature of the component reactions: molecules are fitted together, and move in logical sequences, like the parts of a well-made engine.

The molecular revolution has also altered the nature of the biological community. The spread of its techniques and language has broken down former barriers between disciplines, and its approach has encouraged a brutally direct search for the mechanisms underlying many complex phenomena. The recombinant DNA breakthrough will increase this trend.

It seems probable that relatively few major, universal rules of cell organization still await discovery; and with the molecular revolution thus subsiding, molecular biology seems to be losing its identity. In one direction, it is being absorbed into biochemistry and biophysics, as phenomena already known in outline are being examined in ever greater chemical and physical detail. In another direction, it is being integrated into the field of cell biology.

In addition, increasingly specialized systems, with novel features superposed on the basic cellular models, are being studied. We can be confident that the millions of species, and their innumerable differentiated cells, will furnish a virtually endless frontier (as will also the problems of developmental biology and of neurobiology). And while the analysis of specialized phenomena lacks the particular excitement of discovering a principle of the widest generality, we must also recognize that diversity is a fundamental feature of biology: it was the main interest of natural historians working at a visible level, and it is now being explored at other levels. Moreover, this approach has particular relevance for applications: applied biology inevitably deals with concrete products of diversity.

After a period in which biochemical studies on small molecules yielded many immediately useful products (for example, nutritional factors, hormones, and antibiotics), the shift to the less accessible intracellular macromolecules failed to yield an equally immediate harvest. Now, after a delay of several decades, the promise of novel large-scale commercial applications seems even greater. This development revives confidence in the argument that a broad program of basic, untargeted research will inevitably have unpredictable practical payoffs and hence deserves public support. In the next decade we may also expect a shift of emphasis from medical to agricultural research, in a world with population outrunning food supplies.

As biology delves more and more into human behavior it may be expected to interact increasingly with other disciplines concerned with understanding man. In this connection it should be emphasized that the reductionism of biology is only a heuristic device, deepening our understanding of any level of organization by relating it to other levels: the purpose is not to replace one level by another. In particular, the triumph of the mechanistic approach at the molecular

level in no way reduces our appreciation of the unique intellectual, moral, and esthetic capacities of our species. Rather, we can only stand in awe that life, evolving from inorganic matter, could develop these qualities.

We should also note the increasing implications of biology for philosophy. A modern approach to the classical problems of epistemology should surely take into account the perception that evolution has provided us with a great deal of information encoded in our genome, and this information, expressed in part in our brain structure (and thus justifying the Kantian a priori categories), interacts with personal experience to give us our knowledge of the external world. Advances in neurobiology will no doubt eventually provide more detailed insights. In another area, the potential impact of sociobiology on ethics has recently aroused much discussion.

Biology is also having an increasing impact on the philosophy of science. The revolution in physics led to the widely accepted view that science makes its main progress not by small steps within a universally accepted conceptual framework or paradigm, but by periodically overturning that paradigm. The revolution in biology, however, has not had this character: instead of destroying paradigms, and overturning previous convictions about mechanisms, it has opened up areas, in an infinitely complex material, where biologists knew that they did not know. In a word, this revolution has overturned only previous limits to our powers of experimental analysis, thereby destroying an almost vitalistic earlier attitude toward complexity.

I would close by briefly commenting on some areas of recent public concern. We have recognized, belatedly, that large-scale technological applications of the physical sciences have costs and dangers as well as benefits. In biology, in contrast, concern has been directed not at present harm but at hypothetical future harm. In particular, recombinant DNA research was seen as a possible source of novel epidemics. The anxiety has now abated considerably: extensive work with recombinants failed to produce any harm, and sober professional analyses displaced unrealistic demands for absolute protection against conjectural risks. A retrospective analysis may help us to create better social mechanisms for utilizing the knowledge and judgment of the scientific community in assessing dangers and benefits, and for involving the general community in ways that serve its interests in reality and not only in appearance.

Genetic engineering has also been a source of concern, on the tacit assumption that if we should learn how to cure monogenic hereditary diseases we could use the same power to modify personalities genetically. On technical grounds, however, the extrapolation seems unwarranted, both because most of the large number of genes contributing to any behavioral trait and because most of them will have made their contribution to individuality before birth. But the most fundamental consideration is that any basic knowledge is double-edged, with both good and bad possible applications—and we cannot foresee these in detail. I would suggest that this principle applies not only to knowledge yielding novel powers to manipulate genes, but also to knowledge about the biological roots of human nature. To be sure, such knowledge often encounters resistance, because its possible contradiction of treasured preconceptions, and even more its possible distortions, can have political consequences. One safeguard is to recognize the limited power of science in this area: it cannot prescribe solutions to moral problems, for these involve value judgments as well as estimates of reality. At the same time, a more accurate perception of reality will surely be helpful. The extent of this help is one of the large uncertainties in the future of biology.

Frontiers in Chemistry

Robert M. Joyce, Editor

Chemistry is a multifaceted science. It provides new substances, ranging from complex organic pharmaceuticals and agricultural chemicals to sophisticated inorganic solids that control the flow of electrons to make computers work. It unravels the intricate atomic combinations that nature has learned over millennia to assemble. It discovers catalysts that make it possible to duplicate and to modify complex natural substances, to control the stereochemistry of reactions at chiral sites, and to convert petroleum and coal into basic chemical compounds. It creates synthetic macromolecules, and is developing a wealth of information about the structures and mechanisms of action of natural biomacromolecules. It probes the intimate details of chemical events that occur in less than 1 nanosecond, and detects atoms and compounds at levels in the picogram range in complex mixtures. It studies reactions of atoms and small molecules in various quantum states. This article describes some of the recent advances in a few of the many areas of chemical science.

Preparation of this article was coordinated by J. Bigeleisen, T. L. Cairns, W. G. Dauben, and W. Spindel, all of the Office of Chemistry and Chemical Technology, National Research Council, National Academy of Sciences, Washington, D.C. 20418. Contributors to the article included E. I. Baucom and D. D. Bly, E. I. duPont de Nemours, Wilmington, Delaware 19898; R. Hoffmann, Cornell University, Ithaca, New York 14853; R. M. Joyce, Hockessin, Delaware; M. Paul, 1772 Horatio Avenue, Merrick, New York 11566; J. A. Stephens, Monsanto Co., St. Louis, Missouri 63166; B. Widom, Cornell University; and R. N. Zare, Stanford University, Stanford, California 94305. Overall editing was done by R. M. Joyce, RD 1, Box 86, Hockessin, Delaware 19707.

Instrumentation and Analytical Chemistry

Analytical instrumentation has progressed markedly in the last few years, spurred on by a variety of technical advances. As often is the case, a few basic inventions have been extended and developed by others to provide a vast array of new and complex analytical systems. For example, semiconductor technology has pervaded nearly all aspects of analytical science, providing the sophisticated electronic instrument controls and readout devices we know today. Digital computers, especially fast and accurate micro- and miniprocessors, and in some cases large number-crunchers or data analyzers, have become essential and integral parts of modern measurement

0036-8075/80/0704-0089$01.50/0